The beneficial therapeutic effects of statins, angiotensin-converting enzyme inhibitors and angiotensin-II receptor blockers on protein-C and protein-S activities in Egyptian patients with type-2 diabetes mellitus

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Objectives
Patients with type-2 diabetes mellitus (T2DM) have an increased incidence of adverse cardiovascular events secondary to endothelial dysfunction, hypercoagulability, and decreased fibrinolysis. This study aimed to evaluate protein-C and protein-S activities and carotid intima-media thickness (CIMT) in patients with T2DM who were treated with statins and/or angiotensin-converting enzyme inhibitor (ACEI)/angiotensin-II receptor blockade (ARB).

Basic methods
One hundred and twenty patients with T2DM participating in the study were classified into groups based on their use of statins and ACEI/ARBs. Protein-C and protein-S activity and CIMT were compared.

Main results
Patients treated with both statins and ACEI/ARBs showed the highest levels of protein-C and protein-S activity ($P < 0.001$). This was followed by patients on statins alone and patients on ACEI/ARBs alone. Patients who were not on statin or ACEI/ARB therapy had the lowest levels of protein-C and protein-S activity. Moreover, we identified significant correlations between protein-C and protein-S activities and CIMT with hemoglobin A1c, cholesterol, and low-density lipoprotein.

Conclusion
ACEI/ARBs and statins have a critical impact on the hypercoagulable state characteristic of T2DM, potentially via increased levels of protein-C and protein-S activity. ACEI/ARBs also limited CIMT, an important surrogate marker for atherosclerosis.

Keywords:
carotid intima-media thickness, diabetes mellitus, Egypt, hypertension, protein C, protein S

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Introduction

Type-2 diabetes mellitus (T2DM) is associated with complex metabolic dysfunction as a result of inadequate insulin secretion, resistance to insulin action, and abnormal secretion of glucagon [1]. Vascular complications of T2DM are among the most serious and lethal manifestations of the disease [2]. Atherosclerosis of coronary, cerebral, and peripheral arteries is the predominant cause of T2DM-associated mortality and accounts for up to 70% of all deaths in patients with diabetes. Most individuals with T2DM experience moderate-to-marked insulin resistance associated with several cardiovascular (CV) risk factors (obesity, dyslipidemia, hypertension, endothelial dysfunction, and hypercoagulant state), which together can result in metabolic syndrome [3]. Efforts to reduce hemoglobin A1c (HbA1c) in patients with T2DM had only a modest effect on limiting the CV complications. By contrast, correction of traditional risk factors associated with cardiovascular disease (CVD) (hypertension and hyperlipidemia), inhibits the risk of disease and disease-related death in patients diagnosed with T2DM [4]. The atherosclerotic process is accelerated in patients with T2DM via protein glycation and the synthesis of advanced-glycation end products that are involved in each step of the atherosclerotic process, notably via their roles in promoting clot formation via activation of the coagulation cascade [5].

Parallel to the atherosclerotic complications, patients with T2DM have a propensity to develop thrombosis. Patients with diabetes demonstrate increased expression of glycoprotein IIB/IIIA and von Willebrand factor, both of which promote platelet activation. The fibrinolytic system is inhibited by activated plasminogen-activator inhibitor in patients

with T2DM, this results in accelerated generation of plaque and increased thrombus formation. Also, patients with T2DM have decreased levels of circulating anticoagulants, including protein C and antithrombin, and elevated concentrations of coagulation factors II, V, VII, VIII, and X. Taken together, these factors favor the development of macrovascular complications in association with T2DM [6]. Physiologically, insulin decreases thrombosis and enhances fibrinolysis; in T2DM, insulin resistance creates a prothrombotic state. Furthermore, decreased insulin levels lead to accumulation of calcium in platelets, thereby enhancing platelet aggregation [7].

Protein C as well as protein-C cofactor, protein S, degrade activated FVIII and FV on the surface of negatively charged phospholipid membranes, thus suppressing coagulation [8]. These factors promote fibrinolysis by downregulating thrombin as well as by reducing the concentrations of plasminogen-activator inhibitor-1 and thrombin-activated fibrinolytic inhibitor [9]. Similarly, there is an inverse relationship between the plasma levels of protein C and platelet-derived growth factor (PDGF). PDGF may promote vascular remodeling and atherosclerosis by chemotaxis and proliferation of myofibroblasts and smooth muscle cells, as well as via the production of extracellular matrix components and adhesion molecules. Decreased production of protein C in T2DM promotes formation and release of PDGF from endothelial cells and macrophages [10]. Likewise, inflammation is counteracted by the cytoprotective effects of protein C that are governed by the endothelial protein-C receptor and protease-activated receptor-1 [9].

Multifactorial interventions promote improvements with respect to CV risk factors and mortality associated with T2DM, these include strict control of both blood pressure and blood sugar, stop smoking, healthy dietary habits, exercising, treatment of dyslipidemia [11], and efforts to decrease carotid intima-media thickness (CIMT), which represents a pivotal indicator of early atherosclerosis that is related to thrombotic events [3].

Close monitoring of blood pressure has been proposed as a means to reduce the CV risk associated with T2DM. Antihypertensive drugs have direct vasoprotective effects, their use may serve to reduce vascular complications in patients with T2DM and concomitant hypertension [12]. Angiotensin-converting enzyme inhibitor (ACEI) and angiotensin-II receptor blockade (ARB) are first-line treatment of high blood pressure in patients with T2DM, hypertension, an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m^2, and a urinary albumin–creatinine ratio more than or equal to 300 mg/g creatinine; these agents are used as they can also prevent chronic kidney disease [13]. ACEIs/ARBs also possess antiproliferative, antiatherosclerotic, antiarrhythmic, and antithrombotic effects and have a positive impact on hemostasis, endothelial function via a decrease in the thrombin synthesis, and elevated levels of protein C in patients diagnosed with T2DM [14]. Statins are lipid-lowering and vasoprotective drugs that improve the endothelial function, stabilize the atherosclerotic plaques, and promote anti-inflammatory and antithrombotic effects. These actions may be related to their capacity to inhibit synthesis of isoprenoid intermediates of the mevalonate pathway [15,16].

Patients and methods

The current study included 120 patients diagnosed with T2DM. Patients were recruited from the Endocrinology Outpatient clinic of the Department of Internal Medicine. The participants were subdivided into four groups based on their current drug regimen, including group A, 30 patients undergoing treatment with ACEI/ARBs; group B, 30 patients undergoing treatment with statins; group C, 30 patients undergoing treatment with both statins and ACEI/ARBs; and group D (control group), 30 patients not undergoing treatment with either ACEI/ARBs or statins. Written consents were signed by all study participants before participation in this study. The study was approved by the Department of Internal Medicine as well as the Ethical Committees of the Clinical Pathology Department.

The patients' age ranged from 30 to 60 years. Patients with other known causes of diabetes, who were on anticoagulant therapy, with an eGFR less than 60 or a hepatic disease associated with a Child–Pugh score of class B or C, were exempted from this work.

Study participants were subjected to full medical history, complete physical examination, including a calculation of BMI, and laboratory evaluations that included a blood count, fasting and 2-h postprandial blood glucose and HbA1c levels, renal function tests (creatinine, uric acid, and albumin/creatinine ratio), lipid profile (total cholesterol and triglycerides), coagulation profile [prothrombin time, PC, and international normalized ratio (INR)], protein-C and protein-S activities, and CIMT using carotid duplex imaging as described below.

Carotid duplex imaging. Imaging was performed in order to measure intima-media thickness (CIMT)

using B-mode grayscale, color, and spectral Doppler techniques. The common, internal, and external carotid arteries and the carotid bulbs were examined bilaterally for atherosclerotic plaque, which was defined by localized extension into the arterial lumen with a more than or equal to 50% thickness more than the surrounding wall thicknesses. The number, location, and sonographic appearance of the plaques were recorded. CIMT of the far wall of the distal common carotid artery was measured 1 cm proximal to the flow divider and at end diastole using automated QLab software (Philips Medical Systems, 450 Old Niskayuna Road, Letham, NY 12110 USA). IMT was not measured at the level of a plaque. The results are presented as the mean of 3 values from both the left and right segments [17]. A single radiologist interpreted all study results and was blinded with regard to the patient demographics, medications, and whether they had undergone any previous ultrasound studies.

Evaluation of functional protein-C and protein-S activity. Two milliliters were withdrawn aseptically from all the study participants, venous blood was mixed with sodium citrate anticoagulant (3.2%) at a ratio of 9: 1. Samples were centrifuged twice at 3000 rpm for 10 min at room temperature in order to separate platelet-poor plasma. Samples were collected and stored at less than or equal to −20°C prior to evaluation. In preparation for analysis, samples were allowed to thaw at 37°C for 10 min and were assayed quantitatively by chromogenic evaluations for protein-C and protein-S activities using Berichrom protein-C and protein-S Ac assays, respectively (Siemens Healthcare Diagnostics Products GmbH, Emil-von-Behring-Strasse 76, 35041 Marburg, Germany) on the Sysmex CS-5100 automated coagulation analyzer. Two controls that were processed as though they were test samples were evaluated with each calibration and during testing each day. The coefficient of variation from day to day was 0.4% for protein C and 1.9–7.7% for protein S. Expected values for protein C and protein S were 70–140% and 60–130%, respectively.

Statistical analysis

Data were coded and entered using the Statistical Package for the Social Sciences (SPSS), version 25 (IBM Corp., Armonk, New York, USA). The data were presented using mean and SD for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Multiple comparisons were performed with analysis of variance with a post-hoc test for normally distributed quantitative variables; nonparametric Kruskal–Wallis and Mann–Whitney tests were used for nonnormally distributed quantitative variables [18]. For comparing categorical data, χ^2 tests were performed. Fisher's exact test was used when the expected frequency is less than 5 [19]. *P* values less than 0.05 were considered statistically significant.

Results

The study comprised 120 patients diagnosed with T2DM who were further subdivided into four groups. Demographic and laboratory data, as well as measurements of protein-C and protein-S activities and CIMT for each of the four groups, are as shown in Table 1.

Patients in group C (treated with both statins and ACEI/ARBs) had the highest levels of protein-C and protein-S activities when compared with the other groups; those in group B (statins only) had the next-highest values, followed by those in group A (ACEI/ARBs only). Protein-C and protein-S activities were substantially lower among those in group D. There were also significant differences with respect to age ($P < 0.001$), mean BMI ($P < 0.001$), serum cholesterol ($P < 0.001$), low-density lipoprotein (LDL) ($P = 0.046$), and triglycerides ($P = 0.026$). As above, there were also significant differences with respect to protein-C ($P < 0.001$) and protein-S ($P < 0.001$) activities. By contrast, there were no significant relationships with respect to the duration of illness (T2DM), blood glucose levels either when fasting (FBS) or at 2 h postprandial (2 h PP), or levels of HbA1c, creatinine, uric acid, ACR, eGFR, INR, and CIMT (Table 1). The differences reported above were evaluated among pairs of study groups. Among these, there were significant differences in patient age when comparing groups A and D ($P = 0.003$), groups B and D ($P = 0.001$), and groups C and D ($P < 0.001$). With respect to high blood pressure, there were significant differences between groups A and B ($P = 0.001$), groups A and C ($P = 0.001$), and groups A and D ($P = 0.001$). Similarly, with respect to BMI, significant differences were observed between groups A and D ($P = 0.008$), groups A and C ($P < 0.001$), and groups B and C ($P = 0.004$). With respect to serum cholesterol levels, there were significant differences between groups A and C ($P = 0.001$) and groups B and C ($P < 0.001$). Moreover, with respect to protein-C activity, significant differences were observed between groups A and D ($P < 0.001$), groups A and C ($P < 0.001$), groups A and B ($P < 0.001$), groups B and D ($P < 0.001$), and groups C and D ($P < 0.001$). Finally, with respect to protein-S activity, significant differences were observed between groups A and D ($P = 0.003$), groups B and D ($P < 0.001$), and groups C and D ($P < 0.001$) (data not shown).

Table 1 Comparison of demographic and laboratory data among the patients' groups

	Group A (*N*=30) (ACEI/ARBs only)	Group B (*N*=30) (statins only)	Group C (*N*=30) (ACEI/ ARBs and statins)	Group D (*N*=30) (no ACEI or statins)	*P*
Sex [*n* (%)]					
Male	14 (46.7)	21 (70)	20 (66.7)	16 (53.3)	–
Female	16 (53.3)	9 (30)	10 (33.3)	14 (46.7)	–
Hypertension [*n* (%)]	9 (30)	30 (100)	11 (36.6)	14 (46.7)	–
Age (years) (mean±SD)	54.27±4.74	55.23±3.18	55.27±3.97	49.40±7.8	<0.001
BMI (%) (mean±SD)	24.70±1.02	25.43±1.3	26.6±1.5	25.8±1.3	<0.001
Duration of DM (years) (mean±SD)	5.33±4.49	4.1±2.43	6.3±3.51	4.4±3.17	0.067
FBS (mg/dl) (mean±SD)	140.53±45.77	142.23±56.3	129.6±39.28	124.13±33.03	0.332
2-h pp (mg/dl) (mean±SD)	197.57±48.95	210±67.15	186.67±45	176.73±48.52	0.094
HbA1c (%) (mean±SD)	7.56±0.75	7.49±0.87	7.24±0.69	7.51±0.65	0.353
Creatinine (mg/dl) (mean±SD)	0.86±0.19	0.89±0.19	0.9±0.19	0.93±0.22	0.585
Uric acid (mg/dl) (mean±SD)	5.97±1.6	6.02±1.09	5.85±1.07	6±1.36	0.957
ACR (μg/mg creatinine) (mean±SD)	93.06±101.58	79.62±90.48	114.78±95.07	94.16±105.31	0.582
eGFR (ml/min/1.73 m^2) (mean±SD)	89.43±26.54	93.23±30.73	90.57±27.68	86.27±30.29	0.826
Cholesterol (mg/dl) (mean±SD)	207.83±30.21	210.63±27.06	160.13±50.81	192±68.35	<0.001
LDL (mg/dl) (mean±SD)	73.83±17.56	68.87±12.25	62.97±10.66	73.2±22.87	0.046
Triglycerides (mg/dl) (mean±SD)	113.7±67.81	108.8±22.22	137.67±32.5	138.77±55.88	0.026
INR (mean±SD)	0.99±0.2	1±0.02	1.03±0.06	1.05±0.08	0.238
CIMT (mean±SD)	0.86±0.23	0.96±0.21	0.86±0.19	0.84±0.25	0.154
Protein C (%) (mean±SD)	84.91±15.66	107.2±22.64	119.43±21.25	47.53±16.38	<0.001
Protein S (%) (mean±SD)	80.15±16.24	86.35±12.86	89.32±16.62	64.89±19.09	<0.001

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor blockade; CIMT, carotid intima-media thickness; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HbA1, hemoglobin A1c; INR, international normalized ratio; LDL, low-density lipoprotein.

Among all patient groups, protein-C and protein-S activities were significantly correlated with levels of HbA1c (P < 0.001 and P = 0.014, respectively), serum creatinine (P = 0.045 and 0.014, respectively), cholesterol (P = 0.011 and 0.037, respectively), LDL (P = 0.001 and 0.037, respectively), and INR (P = 0.029 and 0.022, respectively). Moreover, protein-S activity was significantly correlated to GFR (P = 0.012).

CIMT correlated significantly with age (P < 0.001), BMI (P < 0.001), duration of illness (P < 0.001), FBS (P = 0.019), 2-h PP (P = 0.003), HbA1c (P < 0.001), serum creatinine (P < 0.001), uric acid (P < 0.001), cholesterol (P < 0.001), LDL (P = 0.014), ACR (P < 0.001), eGFR (P < 0.001) (Table 2), and hypertension (P = 0.03) (data not shown).

Among patients in group A, protein-C activity correlated significantly to protein-S activity (P = 0.022), CIMT (P < 0.001), age (P < 0.001), duration of DM (P < 0.001), FBS (P = 0.010), 2-h PP (P = 0.016), HbA1c (P = 0.005), serum creatinine (P = 0.016), uric acid (P = 0.02), cholesterol (P = 0.001), LDL (P = 0.002), ACR (P < 0.001), and eGFR (P = 0.027). Similarly, protein-S activity correlated significantly with protein-C activity (P = 0.022), CIMT (P = 0.046), and BMI (P = 0.018). CIMT correlated significantly with protein-C activity (P < 0.001), protein-S activity (P = 0.046), age (P < 0.001), duration of illness (P < 0.001), FBS (P = 0.009), 2-h PP (P = 0.018), HbA1c (P = 0.002), serum creatinine (P = 0.005), uric acid (P < 0.001), cholesterol (P < 0.001), LDL (P = 0.001), ACR (P < 0.001), and eGFR (P = 0.001).

Among patients in group B, protein-C activity correlated significantly to hypertension (P = 0.016), BMI (P = 0.012), duration of illness (P = 0.002), FBS (P = 0.001), 2-h PP (P < 0.001), HbA1c (P = 0.003), LDL (P = 0.018), and ACR (P < 0.001). CIMT correlated significantly with BMI (P = 0.006), duration of illness (P < 0.001), FBS (P < 0.001), 2-h PP (P < 0.001), HbA1c (P < 0.001), serum creatinine (P = 0.002), uric acid (P = 0.013), cholesterol (P < 0.001), LDL (P < 0.001), and triglycerides (P < 0.001).

Among patients in group C, protein-C activity correlated significantly with protein-S activity (P = 0.004) and cholesterol (P = 0.044); likewise, protein-S activity correlated significantly with protein-C activity (P = 0.004). CIMT correlated significantly with hypertension (P = 0.002), age (P < 0.001), BMI (P = 0.001), duration of illness (P < 0.001), FBS (P < 0.001), 2-h PP (P < 0.001), HbA1c (P < 0.001), cholesterol (P = 0.009), LDL (P = 0.003), triglycerides (P = 0.036), ACR (P = 0.009), and eGFR (P = 0.005).

Among patients in group D, protein-C activity correlated significantly with protein-S activity (P < 0.001), CIMT (P = 0.008), age (P = 0.021), HbA1c (P = 0.013), and triglycerides (P = 0.022). Similarly, protein-S activity correlated significantly with

Table 2 Correlations between protein-C and protein-S activities and carotid intima-media thickness with demographic and clinical parameters in all groups

	CV (*P*)		
	Protein C	Protein S	CIMT
Age (years)	0.103 (0.263)	0.012 (0.893)	0.620 (<0.001)
BMI (%)	−0.030 (0.745)	−0.001 (0.994)	0.358 (<0.001)
Duration of DM (years)	−0.065 (0.483)	−0.020 (0.826)	0.673 (<0.001)
FBS (mg/dl)	−0.179 (0.05)	−0.131 (0.155)	0.505 (<0.001)
2-h pp (mg/dl)	−0.103 (0.263)	−0.093 (0.312)	0.514 (<0.001)
HbA1c (%)	−0.359 (<0.001)	−0.224 (0.014)	0.556 (<0.001)
Creatinine (mg/dl)	−0.183 (0.045)	−0.224 (0.014)	0.543 (<0.001)
Uric acid (mg/dl)	−0.148 (0.107)	−0.176 (0.054)	0.571 (<0.001)
Cholesterol (mg/dl)	−0.231 (0.011)	−0.190 (0.037)	<0.475 (0.001)
LDL (mg/dl)	−0.324 (<0.001)	−0.232 (0.011)	0.544 (<0.001)
Triglycerides (mg/dl)	−0.058 (0.527)	−0.044 (0.635)	0.167 (0.167)
INR	−0.200 (0.029)	−0.209 (0.022)	0.160 (0.082)
ACR (µg/mg creatinine)	−0.126 (0.171)	−0.066 (0.472)	0.520 (<0.001)
eGFR (ml/min/1.73 m^2)	0.177 (0.054)	0.230 (0.012)	−0.501 (<0.001)

CIMT, carotid intima-media thickness; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HbA1, hemoglobin A1c; INR, international normalized ratio; LDL, low-density lipoprotein.

protein-C activity ($P < 0.001$) and HbA1c ($P = 0.042$). CIMT correlated significantly with protein-C activity ($P = 0.008$), hypertension ($P = 0.003$), age ($P < 0.001$), duration of illness ($P < 0.001$), FBS ($P = 0.019$), 2-h PP ($P = 0.003$), HbA1c ($P < 0.001$), serum creatinine ($P < 0.001$), uric acid ($P < 0.001$), LDL ($P < 0.001$), INR ($P = 0.014$), ACR ($P < 0.001$), and eGFR ($P < 0.001$).

Discussion

Accelerated atherosclerosis is a key feature of diabetic CV complications and is the main cause of death. Patients with T2DM are 10 times more likely to develop CVD in their lifetime [20]. Diabetes has a profound impact on blood vessel endothelial cells, smooth muscle cells, platelets, lipoproteins, local production and function of vasoactive substances, and clotting factors; diabetes also has significant impact on local arterial responses to hypoxia and formation of new collateral vessels [21]. Concentrations of coagulation factors VII, VIII, XIII, and von Willebrand factor have been reported as increased, whereas those of antithrombin III and protein C are relatively decreased among those diagnosed with diabetes [22].

The most effective approach for prevention of CVD in association with diabetes is multifactorial risk factor reduction [1]. Statins, ACEIs, and ARBs have been proven to be beneficial not only for the correction of dyslipidemia and control of hypertension, respectively, but also for primary and secondary prevention of CVD and death associated with coronary heart disease in diabetic patients [23].

As such, this study aimed to determine the specific impact of statins and ACEI/ARBs on protein-C and protein-S activities as well on CIMT in patients diagnosed with T2DM. In the current study, we found that the patients undergoing combined therapy with statins and ACEI/ARBS had higher levels of protein-C and protein-S activities when compared with those in the other study groups. The next-highest levels were detected among patients undergoing treatment with statins alone, followed by patients on ACEI/ARB therapy. Patients with T2DM who were not treated with either drug had the lowest protein-C and protein-S activities, the differences between these groups were highly significant ($P < 0.001$). These results were in agreement with those by Kim *et al.* [24] and Aktaş *et al.* [14] who studied the effects of ACEI/ARBs and statins on protein-C levels in patients with diabetes. Their results indicated significantly higher levels of protein C among patients with diabetes on drug therapy compared with controls. Similarly, Kim *et al.* [24] found that patients who were undergoing treatment with statins and ARBs had significantly lower levels of hypercoagulability, as indicated by their high protein-C levels; this was followed by patients treated with statin or ARBs, with lowest levels detected in patients who were taking neither of the two drugs. Moreover, Park *et al.* [25] concluded that protein C, protein S, and antithrombin activities were significantly elevated in patients with T2DM who were treated with statins compared with patients who were statin-free. These results suggest that statins are not only lipid-lowering but also have significant anti-inflammatory and antithrombotic effects.

Active protein C has a critical role in coagulation, inflammation, maintenance of blood vessel permeability, and apoptosis of cells. Protein C promotes anticoagulation via its role catalyzing the inhibition of coagulation factors Va and VIIIa, these factors are important for the activation of factor X and thrombin formation. Interestingly, venous thromboembolism is among the cardinal manifestations of protein-C deficiency; there are several published reports that have documented CVDs in young adults with congenital protein-C deficiency with no other evident CV-precipitating factors [26]. The anticoagulant effects of statins were proved in previous researches involving lowering expression of tissue factor associated with reduced generation of procoagulant reactions

catalyzed by thrombin, these include fibrinogen cleavage, factor-V and factor-XIII activation, as well as enhanced endothelial thrombomodulin expression, which ultimately results in protein-C activation and factor-V inhibition [27].

Hypertension as well as insulin resistance contribute mainly to activation of the renin–angiotensin system in patients diagnosed with metabolic syndrome. ACEI therapy in these patients theoretically will result in improved insulin action as well as reductions in CVD. ACEIs have been shown to be more efficient than other antihypertensive medications with respect to reduction of CVD-associated morbidity and mortality in hypertensive diabetics [28].

ACEI therapy has many advantages other than its antihypertensive effects. ACEIs are antiproliferative, antiatherosclerotic, and antiarrhythmic and have positive effects on endothelial function and hemostasis [29].

In this study, high levels of HbA1c suggestive of poor control of blood glucose, correlated with decreased activity of the naturally occurring anticoagulants, protein C and protein S, in patients with T2DM. Patients with persistently high HbA1c levels have lower levels of protein-C and protein-S activity and increased CIMT compared with those patients whose blood glucose levels were adequately controlled. This finding suggests that poor glycemic control may be directly associated with the characteristic hypercoagulable state in T2DM. Sustained hyperglycemia results in nonenzymatic glycation of proteins (including protein C and protein S), resulting in high levels of AGEs that have been directly associated with the development of atherosclerosis and long-term diabetic complications [5]. Glycosylation of protein C and protein S decreases their respective activities and thus predisposes a given T2DM patient to an increased risk of thrombosis [30].

In this study, we found that CIMT correlated significantly with age, BMI, ACR, lipid profile, the duration and control of T2DM, and the specific therapeutic regimen. Our findings were in agreement with those by Kota *et al.* [31] who studied the CIMT in patients diagnosed with T2DM and healthy individuals. CIMT was significantly higher among the T2DM patients and was significantly related to other CV risk factors, including older age, elevated blood pressure, higher lipid levels, lower high-density lipoprotein cholesterol, glycemic control, and the duration of illness (T2DM). CIMT is widely used as a marker and an important means for noninvasive evaluation of atherosclerosis and CVD, it represents an essential indicator of future CV events and has been related to CV risk markers, including age, diabetes, obesity, insulin resistance, hypertension, and hyperlipidemia. The Atherosclerosis Risk in Communities study focused on individuals aged 45–64 years and proved evident relations of CIMT changes with blood glucose levels, tobacco smoking, high-density lipoprotein cholesterol, pulse pressure, leukocytic count, and fibrinogen levels. Furthermore, glycemic parameters (fasting plasma glucose, postprandial plasma glucose, and HbA1c) and the lipid parameters (total cholesterol, LDL cholesterol, and triglycerides) were all significantly elevated in patients with increased CIMT [32].

The UK Prospective Diabetes Study reported that blood pressure control was found to be of equal importance to control of blood glucose levels in order to limit CV complications in diabetic patients [33,34].

Conclusion and recommendations

Drug regimens for T2DM that include both ACEI/ARBs and statins have pleiotropic beneficial effects. The results of this study revealed the impact of these two drugs on reducing the hypercoagulable state that is a characteristic of T2DM by increasing protein-C and protein-S activities and reducing CIMT as a surrogate marker for atherosclerosis.

Further multicenter studies with larger number of patients will be needed for further evaluation of this therapeutic regimen in T2DM patients with a specific focus on their role in limiting hypercoagulability and CVD-associated complications. Long-term follow-up of these patients is also recommended to identify the groups at highest risk, so that appropriate preventive measures might be applied.

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The paper has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the paper represents honest work.

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Conflicts of interest

There are no conflicts of interest.

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